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Anti-Ulcer Activity of 3-methoxysalicylaldehyde-2-aminobenzoylhydrazone Derivatives in Rats

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ABSTRACT

Peptic ulcer is a one of the major ailment effecting 10% of the population. Drugs are found by screening compounds against an animal model of human disease. Main aim of the present study is to evaluate anti-ulcer activity of cobalt and nickel derivatives of 3-methoxysalicylaldehyde-2-aminobenzoylhydrazone by pylorus ligation method in albino rats. Anti-ulcer activity was compared with standard drug diclofenac sodium. In pyloric ligation induced ulcer model, the studied parameters were gastric volume, pH, total acidity, free acidity, ulcer index and percentage of ulcer protection. In treated groups of nickel and cobalt derivatives total acidity and ulcer index were significantly decreased, gastric pH was increased and there is no significant change in volume of gastric acid secretion, free acidity as compared to control group. Percentage protection increased dose dependently in animal treated with both the derivatives. Group treated with Nickel derivative showed good results as compared to cobalt derivative treated group. These results indicate that both nickel and cobalt quinazolinone derivatives have better potential against ulcer.

Keywords: Quinazolinone, Peptic ulcer, pylorus ligation, ulcer index.

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INTRODUCTION

Peptic ulceration is one of the common disease affecting millions of people. Excessive stress, smoking, chronic alcohol intake, *H. pylori* bacterial infection and chronic usage of non-steroidal anti-inflammatory drugs are main causes of peptic ulcer. Main manifestations include abdominal pain, mucosal bleeding and inflammation in patients[1, 2]. Gastroprotectives and aggressives imbalance results in ulcer [3]. Research advances during last decade have offered new insights in the therapy and prevention of peptic ulceration. Although drug treatment for peptic ulceration has improved in the recent past, the need for better therapy is still prevailing. Various mechanisms to treat ulcers are stimulation of epithelial cell proliferation, blocking apoptosis, promotion of gastro-protection and inhibition of gastric acid secretion. Most of conventional drugs used for treating ulcers are reported with ADRs, and drug interactions[4]. Struggle to develop an orally effective, less toxic drug is principal goal of modern research to alleviate the burden of peptic ulcer. Quinazolin-4-(3H)-one derivatives are deliberated as attention-grabbing moieties since they possess anti-tumour[5], anti-HIV[6], selective oestrogen beta modulator[7], anti-inflammatory[8], anti-bacterial[9], antidepressant[10] activities, anti-arthritic activity[11]. Therefore, present study was steered to assess anti-ulcer effect of cobalt and nickel derivatives of 3-methoxysalicylaldehyde-2-aminobenzoylhydrazone.

MATERIALS AND METHOD

Synthesis and Characterization of Co-AQF

Condensation of 3-methoxysalicylaldehyde with 2-Aminobenzoylhydrazoneto yielded 3-methoxysalicylaldehyde-2-aminobenzoylhydrazone (AQF). Metal complexes of AQF like Ni-AQF, Co-AQF, Cu-AQF, Mn-AQF were Synthesized, characterized by thermal, NMR, UV-Vis, IR, and Mass spectroscopic methods and evaluated for anti-microbial activity[12]. Ni-AQF was also proven to possess anti-inflammatory and anti-arthritic activity[13].

Acute toxicity study

Female swiss-mice (25-30g) were given limit doses of 2000mg/kg and 550mg/kg according to OECD guidelines-425 and observed for 4hrs and then up to 14days. 100% mortality was occurred with higher limit dose. AOT software was used to calculate $LD_{50.}$ According to the results of acute toxicity study the doses of 5mg/kg Body Weight (BW) and 10mg/kg BW were chosen for the experiment.

Animals

Female Sprague-Dawley (SD) rats (150-200gm) were obtained from central animal house of

HSK College of Pharmacy, Bagalkot maintained with standard husbandry conditions (Temp. 22-28^{0}C; Relative Humidity 65±10%) for 12hr dark and 12hr light cycle respectively in standard propylene cages. The animals were fed with standard food (Pranav Agro Industries, Sangli and Maharashtra) and water *ad libitum*. Experiments were conducted in accordance with Institutional Animals Ethics Committee (HSKCOP/IAEC, Clear/2010-11/1-14).

Chemicals and Instruments

Sodium hydroxide (SD Fine Chemicals Ltd, Mumbai), Oxalic acid, (Qualigene Fine Chemicals, Mumbai), Topfers and Phenolphthalein reagent (Nice Chemicals Pvt. Ltd, Cochin) and Research centrifuge (Remi instruments, Mumbai). All other chemicals and reagents were procured are research grade.

Effect of Ni-AQF and Co-AQF on ulcer index by pylorus ligation method.

SD female rats (150-200gm) divided into 6 groups with 6 in each. Group I as control receiving 10%v/v tween80; p.o., Group II received Diclofenac sodium (5mg/kg; p.o.), Group III received Ni-AQF 5mg/kg, Group IV received Ni-AQF 10mg/kg, Group V received Co-AQF 5mg/kg and Group VI received Co-AQF 10mg/kg once daily for 3 days. On fourth day, pylorus was ligated in 36hr fasted animals[14, 15]. After 4hr of pyloric ligation, animals sacrificed, abdomen was opened and esophageal end of stomach was tied. Thereafter remove stomach, collect gastric fluid open it along greater curvature and wash in DW. Ulcer index was assessed by observing stomach placed on clean glass slide under microscope. Scoring of ulcers is as follows: 0= Normal coloured stomach, 0.5= red coloration, 1= spot ulcers, 1.5= hemorrhagic streaks, 2= Ulcer≥3 but≤5 and 3= ulcers>5 [16].

Effect of Ni-AQF and Co-AQF effect on Gastric secretion, Gastric pH, Acidity in pylorus ligated animals

Gastric content volume was measured and then centrifuged at 1000rpm for 10min. Pipette out 1.0ml of supernatant liquid, dilute it to 10ml with DW and measure pH by pH meter[17]. Than titrate against 0.01N NaOH with Topfer's reagent as indicator and end point turns to orange colour, volume of NaOH corresponds to free acidity. Titrate further till the solution regains pink colour and volume of NaOH corresponds to total acidity and acidity (mEq/l/100g) can express as, Acidity= (vol. of NaOH* Normality of NaOH*100)/0.1

Statistical Analysis

Results were expressed as Mean± SEM. Statistical comparison was made between treated groups and control group. Statistical difference between two means was determined by one-way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism 6.0 software.

Only those mean values showing statistical difference $p<0.001$, $p<0.01$, $p<0.05$ were considered as statistical significant and $p>0.05$ was considered as non-significant.

RESULTS AND DISCUSSION

Effect of Ni-AQF and Co-AQF on Gastric secretion, Gastric pH, acidity, ulcer index and % ulcer protection in pylorus ligated animals

In pylorus ligation induced gastric ulcer model, Ni-AQF and Co-AQF quinazolinone derivatives has shown significant decrease in total acidity ($p<0.001$), ulcer index($p<0.001$) and raised gastric pH ($p<0.001$) and there is no significant change in volume of gastric acid secretion, free acidity as compared to control group. The control group showed increased ulcer index whereas test compounds exhibited significant reduced ulcer index, Ni-AQF 5 mg/kg ($p<0.001$), Ni-AQF 10 mg/kg ($p<0.001$), Co-AQF 5mg/kg ($p<0.001$) and Co-AQF (10 mg/kg) has not shown any significant result as compared to control group. The percentage of ulcer protection showed by Diclofenac sodium is 74.41%, Ni-AQF 5mg/kg is 79.80% ($p<0.01$), Ni-AQF 10 mg/kg is 90.39% ($p<0.001$) and Co-AQF 10mg/kg is 47.10% ($p<0.01$) and Co-AQF 5 mg/kg has not shown any significant result as compared to control group. The ulcer index of the test compounds reveal that, ulcer protectivity of Ni-AQF showed dose dependent action (Table 1). Chemistry of quinazolinone compounds has been the subject of considerable interest though there had been scattered reports of investigation of the medicinal properties of such compounds. Causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid[18]. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. The test compounds Ni-AQF and Co-AQF showed significant ulcer protection by decreasing the total acidity indicating its anti-secretory effect.

Table 1: Effect Co-AQF on Gastric secretion, Gastric pH. Acidity ulcer index and %ulcer protection in pylorus ligated animals.

Groups	**Volume of gastric secretion (ml/100gm)**	**pH of gastrtic juice**	**Free acidity (mEq./l/100g)**	**Total acidity (mEq./l/100g)**	**Ulcer Index**	**% of ulcer protection**
Control(10% tween80)	4.833±0.295	4.333±0.210	18.33±8.751	45.00±5.477	4.333±0.698	----
Diclofenac sodium(5 mg/kg)	4.533±0.091 ns	4.452±0.105 ns	21.67±3.801 ns	50.00±1.461 ns	1.102±0.303***	74.41%
NI-AQF (5 mg/kg)	5.900±0.666 ns	5.667±0.210***	34.67±2.951 ns	26.67±3.273***	0.875±0.285***	79.80%
NI-AQF (10 mg/kg)	5.367±0.264 ns	5.867±0.084***	39.33±2.940 ns	18.33±1.726***	0.416±0.105***	90.39%
CO-AQF (5 mg/kg)	4.500±0.159 ns	6.333±0.105***	32.33±3.393 ns	20.00±1.826***	3.500±0.120 ns	19.22%
CO-AQF (10 mg/kg)	5.067±0.423 ns	6.167±0.105***	34.33±1.054 ns	12.00±0.730***	2.292±0.139***	47.10%

All values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; *p<0.05, **p<0.01, ***p<0.001 as compared to control group; ns=non-significant

CONCLUSION

These results suggested protective role of Ni-AQF and Co-AQF in pylorus ligated peptic ulcer model. Antiulcer activity of Ni-AQF and Co-AQF may be attributed to anti-secretory, cytoprotective and antioxidant properties. Further, studies are required to elucidate the detail mechanism of actions of these agents at molecular level to explore the therapeutic benefits.

CONFLICT OF INTEREST

Authors report no conflict of interest. Authors alone are responsible for the content and writing the article. This study was supported by research grant VGST/P8/CISE/2011-12/1151 from vision group on science and technology, Department of IT, BT, Science & technology, Govt. of Karnataka, Bangalore, and Karnataka, India.

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